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The effect of saline-alkaline and water stresses on water use efficiency and standing biomass of *Phragmites australis* and *Bolboschoenus planiculmis*

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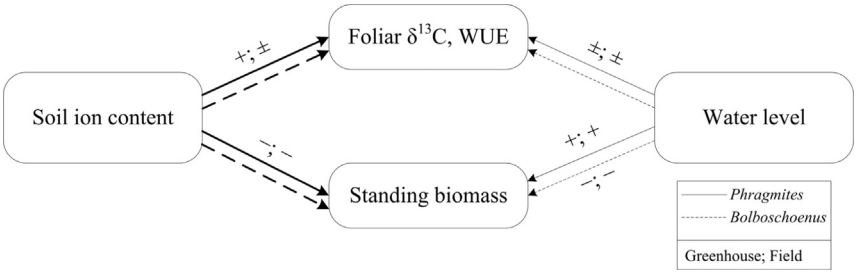
HIGHLIGHTS

- WUE increased with saline-alkaline stress of the two wetland plants.
- Standing biomass was lower with higher stress, independent of water level.
- Impacts of alkaline stress were the largest among all stress types texted.
- Under high ion contents, *P. australis* is rather tolerant to flooding stress.
- In contrast, *B. planiculmis* is rather tolerant to drought stress.

GRAPHICAL ABSTRACT



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ABSTRACT

Salt marsh plants in the West Songnen Plain, northern China, are threatened by increasing soil salinity and alkalinity since the late 20th century. To explore how these wetland ecosystems respond to such environmental changes, we examined the effect of saline-alkaline stresses and water stress (flooding/drought) on water use efficiency (WUE, assessed with stable carbon isotopes) and standing biomass of *Phragmites australis* and *Bolboschoenus planiculmis* under both greenhouse and field conditions. In the field, sodium bicarbonate ($NaHCO_3$) was the main saline-alkaline component, and the soil total ion content was negatively related to water level. Higher soil ion content decreased standing biomass of *P. australis* and *B. planiculmis* in the field and greenhouse, and increased WUE in the greenhouse. With higher water level, standing biomass of *P. australis* increased, while that of *B. planiculmis* decreased in both the field and greenhouse. Alkaline stress exerted the greatest negative influence on growth of *P. australis*, but only under high ion content. Low alkaline ion content promoted growth of *B. planiculmis*. Soil ion content exerted the strongest influence on foliar $\delta^{13}C$ (and thus WUE) and standing biomass of both species compared to water level and stress type. Our findings suggest that under high ion contents, *P. australis* is more tolerant to flooding stress while *B. planiculmis* is more tolerant to drought stress. Moreover, *P. australis* has a high ability to modulate and increase WUE to resist its adverse environment. Our study will contribute to a better understanding of the influence of climate change and increasingly serious human disturbances on the distribution and productivity of these two important wetland species.
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1. Introduction

The intensity of environmental stress, such as intense rainfall, temperature extremes, drought, salinity and alkalinity, is predicted to increase under climate change and intensive land-use change (Flato et al., 2013; Lehner et al., 2006; Liu et al., 2005). Saline-alkaline wetlands, distributed in the inland plains, plateaus, and basins of arid or semi-arid regions around the world (Sun et al., 2000), are increasingly affected by increasing soil saline and alkaline stresses, and flooding/drought extremes, which have become the main environmental threat affecting their structure and function (Cañedo-Argüelles et al., 2014; Garssen et al., 2015). A better understanding of the effect of environmental change on the growth of wetland plants would allow to predict how wetland ecosystems and their functioning would respond to future environmental changes and help to protect and manage these vulnerable systems.

Plant water use efficiency (WUE) is a key ecophysiological indicator for the coupling between carbon dioxide and water fluxes of plants (Lu et al., 2007; Tang et al., 2017). Moreover, the variability of WUE can reflect the responses of terrestrial plants to global change (Huang et al., 2015; Huxman et al., 2004; Niu et al., 2011). In inland saline-alkaline marshes, water is a limiting factor because salinity-alkalinity imposes a water deficit by reducing the soil osmotic potential, even though the plants grow in a regularly flooded environment (Munns and Tester, 2008; Verslues et al., 2006). Hence, WUE is an important factor influencing plant distribution and growth in these ecosystems. Biomass production, as the foundation of the food chain, is one of the main ecosystem functions, supplying habitat, forage and shade for wildlife and organic matter inputs to the soil (Dwire et al., 2004). The hazard of soil salinity and alkalinity on wetland plant growth has received considerable attention in the past decade (Gorai et al., 2007; Pagter et al., 2009; Xiao et al., 2016; Zhou et al., 2015). However, almost all the research focused on saline stress. It has been clearly demonstrated that the toxic effect of alkaline stress (high pH soil resulting from $NaHCO_3$ and Na_2CO_3) on grassland plants such as *Aneurolepidium chinense* and *Kochia sieversiana* is more severe than that of saline stress (Shi and Wang, 2005; Yang et al., 2007). Only few studies have been conducted in wetland ecosystems with different combinations of saline, alkaline and saline-alkaline stress, and little effort has been invested to compare field studies and controlled experiments (but see Deng, 2011 for plant height and chlorophyll content).

Flooding regime is a fundamental factor influencing wetland plant communities that are often found in a fluctuating environment subjected to flooding and drought (Lou et al., 2016; Voesenek et al., 2004). On the one hand, flooding adversely influences wetland plants by reducing gas diffusion rates and oxygen solubility in the water, which restrains plant growth (Bouma et al., 2001; Colmer, 2003). Drought, on the other hand, affects growth and metabolism of many plants and is closely related to high osmotic and oxidative stress, which damages cellular structures and macromolecules (Ahmad et al., 2011; Biehler and Fock, 1996; Smirnoff, 1993). Furthermore, interactive effects of flooding/drought cycles and saline stress on plant growth and survival are stronger than the separate effects of flooding and saline stress (Baldwin and Mendelssohn, 1998; Marcar, 1993; Salter et al., 2007). Therefore, we cannot study the effect of saline-alkaline stresses on wetland vegetation without considering the water level.

Phragmites australis is a tall, perennial helophyte with an extensive system of strong rhizomes, which is one of the most widespread and productive plants in the world (Brix and Cížková, 2001). *Bolboschoenus planiculmis* is a herbaceous perennial plant in the sedge family, found in marshes throughout Eurasia (Govaerts et al., 2014). Its tubers are the major food source for a critically endangered bird species - the Siberian white crane (*Grus leucogeranus*) (Hui et al., 2009; Red list, 2017). The Western Songnen plain in northern China is one of the main distribution areas of *P. australis* and *B. planiculmis* marshes. However, due to the impact of global change and human activities, the marsh area has decreased from approximately 6.4×10^4 km^2 in 1954 to 1.6×10^4 km^2 in 2008 (Wang et al., 2011). Although concern about marshes has been increasing over the last decades, few studies have paid attention to the impacts of saline-alkaline stresses on WUE and standing biomass of the dominant plant species (Gorai et al., 2010; Matoh et al., 1988; Pagter et al., 2009).

In this study, we examined the response of WUE and standing biomass of *P. australis* and *B. planiculmis* to different types and severities of saline-alkaline and water stresses under both field and greenhouse conditions to answer the following three questions:

(1) Does increasing saline-alkaline stresses increase WUE and reduce standing biomass, independently of the water level?
(2) Which of the three stress combinations, saline, alkaline and saline-alkaline stress exerts the largest detrimental effect on WUE and standing biomass?
(3) Do *P. australis* and *B. planiculmis* show different adaptive strategies to saline-alkaline and water stresses?

2. Materials and methods

2.1. Study area

The Western Songnen plain (121°31′ to 126°32′ E, 43°57′ to 48°30′ N) in northern China (Fig. 1), located at the confluence of the Songhua River, Second Songhua River and the Nen River, is characterized by a semi-arid temperate continental monsoon climate. The elevation ranges from 110 to 140 m above sea level, and the relative height is 5 to 10 m between flat terrain and depressions. Mean annual precipitation ranges from 350 to 420 mm in the west to 420 to 460 mm in the east. Mean annual evaporation ranges from 1500 to 1900 mm in the west to 1200 to 1600 mm in the east. Mean annual temperature ranges from about 6 °C in the south to about 2 °C in the north (Wang et al., 2009). Saline-alkaline wetlands are one of the main saline-alkaline land types, with an area of 1×10^4 km^2 (Li et al., 2007). Typical marsh plants are *Phragmites australis*, *Bolboschoenus planiculmis* and *Schoenoplectus nipponicus*. The dominant soil types are salinized soil, solonetzic soil, meadow soil, chernozem, chestnut soil, and aeolian soil.

2.2. Experimental design

2.2.1. Field study

We collected samples at a total of 22 sites that were dominated by one of the two species or co-dominated by both of them in August 2014 and 2015 when plants were at their peak standing biomass of the growing season (Fig. 1). For *P. australis*, we had 6 sites in 2014 and 12 sites in 2015, and for *B. planiculmis*, we had 4 sites in 2014 and 5 sites in 2015. We selected different sites for the two years. Standing biomass was harvested in three 0.5 m × 0.5 m plots at each site. At the same time, fully expanded current-year leaves were sampled and soil from 0 to 20 cm depth collected in each plot.

2.2.2. Pot experiment

Rhizomes of *P. australis* and tubers of *B. planiculmis* were collected in May 2015 in the study area. Rhizome sections were regenerated in soil with 5 to 10 cm water level. After a week, uniform shoots of about 10 cm height were selected. The rhizomes of the shoots were cleaned with tap water and then transplanted into pots (40 cm diameter and 55 cm high, five shoots per pot) containing 20 cm of washed sand. Tubers of *B. planiculmis* were soaked in tap water. After 3 days, uniform shoots of 2 to 5 cm were selected for transplantation into pots (14 cm diameter and 13 cm high, five shoots per pot) containing washed sand to a height of 12.5 cm. The pots were put into large containers that were filled with water in order to maintain the required water levels.

The stress treatments consisted of three to four water levels (0, 15 and 30 cm for *P. australis* and − 5, 0, 10 and 20 cm for *B. planiculmis*)

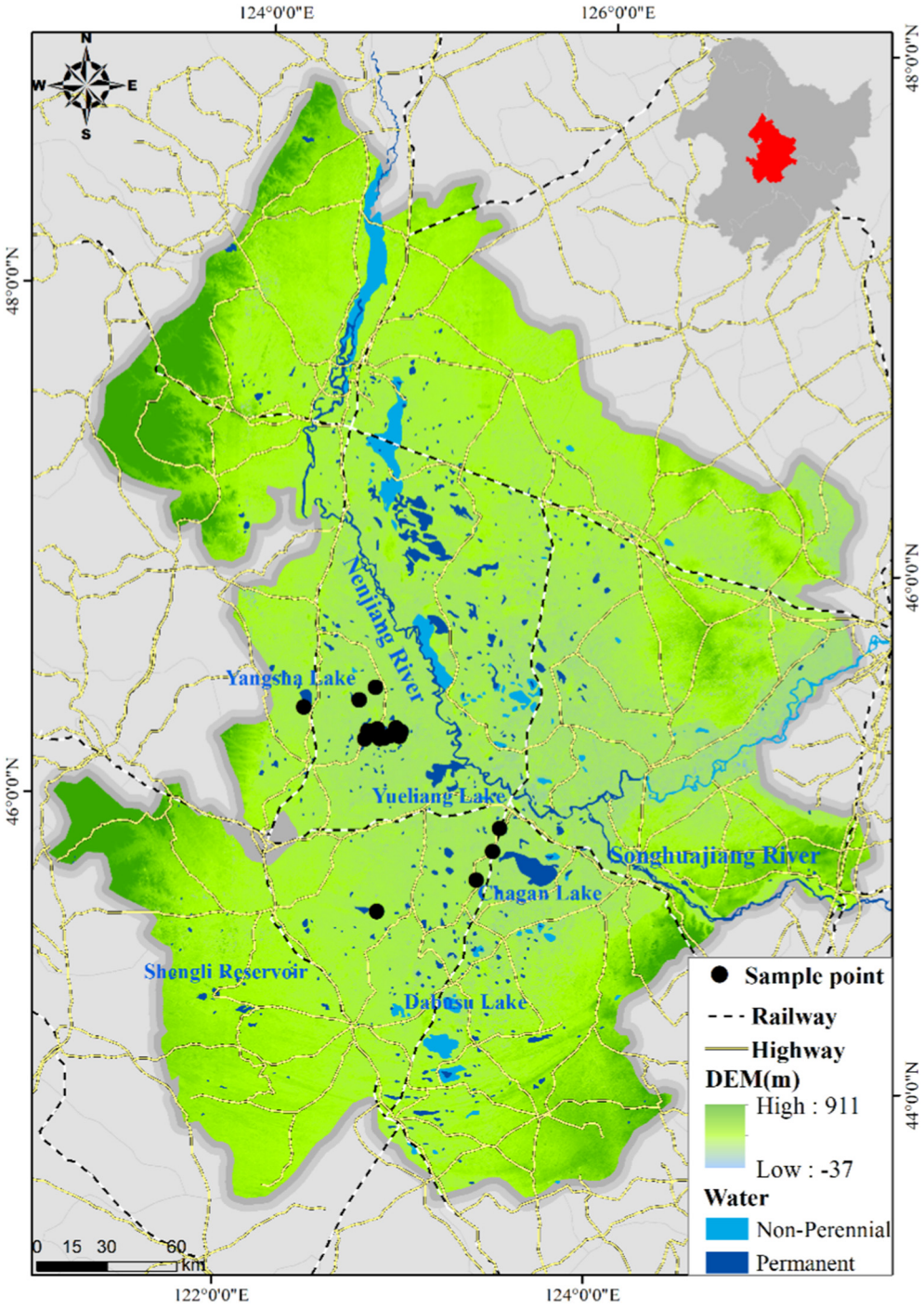

Fig. 1. Location of the West Songnen Plain, Northeast China.

and five ion contents (0, 2.5, 5.0, 10 and 20 g kg^{-1}) of three salt stress types (alkaline stress: $NaHCO_3$; saline stress: NaCl; saline-alkaline stress: $NaHCO_3$ + NaCl). Two basic salts were mixed in a 3:1 mass ratio ($NaHCO_3$: NaCl) and applied to the plants subjected to the saline-alkaline stress. We had 117 observations of *P. australis* and 104 observations of *B. planiculmis* (3 stress types × 4 ion contents plus a "0" ion content, fully crossed with 3 water levels (*P. australis*) or 4 water levels (*B. planiculmis*), replicated 3 times).

The plants were watered with a Hoagland half-strength nutrient solution with added ion content, which originally had the following composition: 0.5 mM $NH_4H_2PO_4$, 3 mM KNO_3, 2 mM $Ca(NO_3)_2{\cdot}4H_2O$, 1 mM $MgSO_4{\cdot}7H_2O$, 23.13 μM H_3BO_3, 4.57 μM $MnCl_2{\cdot}4H_2O$, 0.382 μM $ZnSO_4{\cdot}7H_2O$, 0.16 μM $CuSO_4{\cdot}5H_2O$, 0.0695 μM MoO_3 and 9 μM Fe-EDTA. The nutrient solutions were renewed every week. Mature leaves (for the measurement of $\delta^{13}C$) and standing biomass were fully harvested for further analyses after six and four weeks for *P. australis* and *B. planiculmis*, respectively. The pot experiments were conducted in a greenhouse of Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun.

2.3. Measurements

All plant samples were oven-dried at 70 °C to constant weight for at least 48 h. Dry leaves were ground with a ball-grinding mill (MM400, Retsch, Germany) and weighed into tin capsules for measurements of the carbon isotope ratios ($\delta^{13}C$) with an isotope-ratio mass

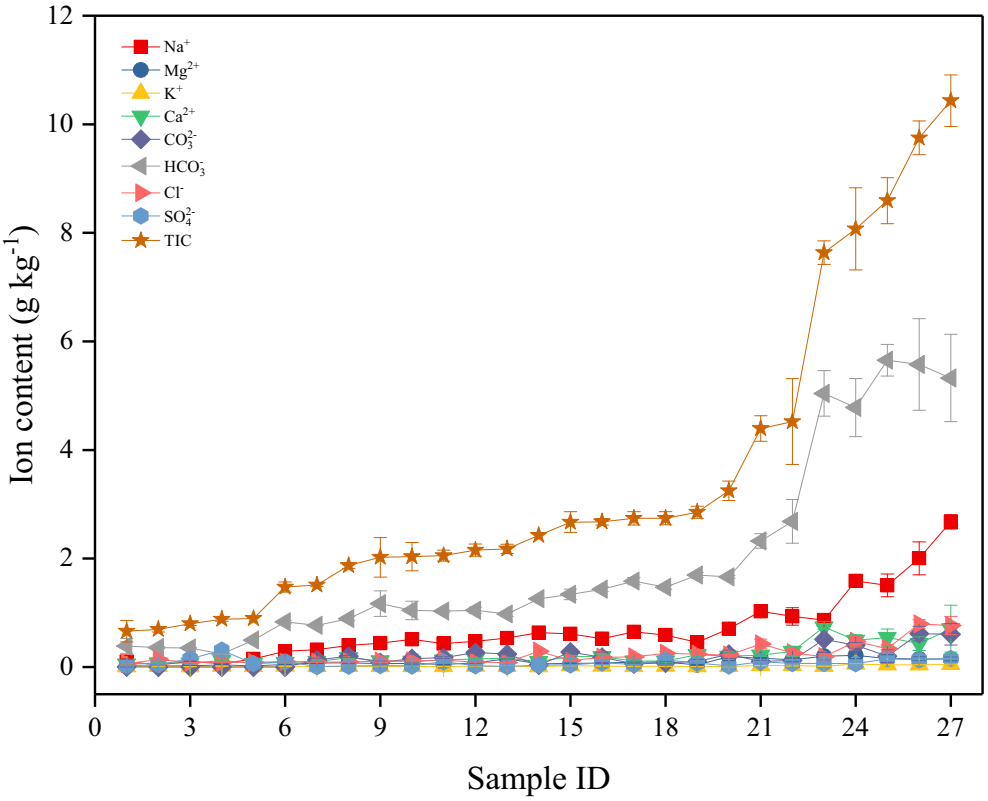

Fig. 2. Mean soil ion contents of Na^+, Mg^{2+}, K^+, Ca^{2+}, CO_3^{2-}, HCO_3^-, Cl^- and SO_4^{2-} as well as mean total ion contents (TIC) from our 22 field sites sampled in 2014 and 2015. Means ± 1 s.e. (n = 3) are shown.

spectrometer (Thermo Finnegan MAT Delta^{plus} XP, 0.15‰, Bremen, Germany).The harvested standing biomass was weighed. The soil was air-dried and then finely ground to pass through 1 mm sieve prior to chemical analysis. HCO_3^-, CO_3^{2-} and Cl^- were measured by the titrimetric method. K^+, Na^+, Ca^{2+}, and Mg^{2+} were analyzed using inductively coupled plasma atomic emission spectrometry (ICPS-7500, Shimadzu Corporation, Japan) with a precision of <10 ppb. SO_4^{2-} was measured by ultraviolet spectrophotometry (UV-2550, Shimadzu Corporation, Japan) with a precision of 0.025 mg.

WUE is a long-term expression of the ratio between carbon assimilation and water loss at the whole plant level (Cernusak et al., 2013). WUE can be calculated using the foliar carbon isotope ratios according to the following formula:

$$WUE = \frac{C_a\left(1-\frac{\delta^{13}C_a-\delta^{13}C_p-a}{b-a}\right)}{1.6(e_a-e_i)}$$

where C_a is the ambient CO_2 concentration, e_i and e_a are the intercellular and atmospheric vapor pressures, a is the discrimination against $^{13}CO_2$ during CO_2 diffusion through the stomata (a = 4.4‰, O'Leary, 1981), b is the discrimination associated with carboxylation (b = 27‰, Farquhar and Richards, 1984), 1.6 is the ratio of diffusivities of water vapor and CO_2 in air, and $\delta^{13}C_a$ (−8.6‰ at San Diego in 33° N, Scripps CO_2 Program, 2017) and $\delta^{13}C_p$ are the carbon isotope ratios in ambient atmospheric CO_2 and of the leaves, respectively.

2.4. Statistical analyses

The relationship between soil ion content and water level was analyzed with a Spearman rank correlation. Interactive effects of soil ion content and water level on foliar $\delta^{13}C$ and standing biomass in the field were tested separately for the two years (2014 and 2015) with linear mixed-effects models using the statistics program R (R Core Team, 2017). Soil ion content, water level and species were considered as fixed effects, while plot was considered as a random effect, nested within the sites (which are represented by the two fixed effects), to correctly represent this spatial variation. For the greenhouse experiment, interactive effects of soil ion content, water level and stress type on foliar $\delta^{13}C$ and standing biomass were tested with linear models using R. For the greenhouse experiment, Tukey *post-hoc* tests were used to

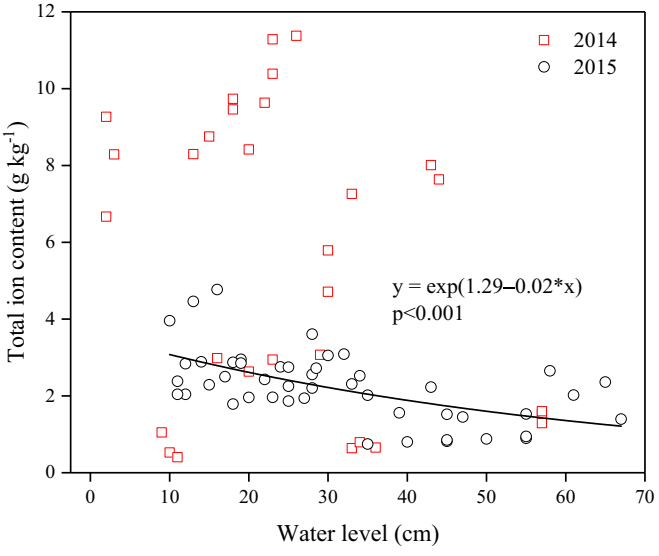

Fig. 3. Relationship between soil total ion content and water level in the field in 2014 (red squares) and 2015 (black circles).

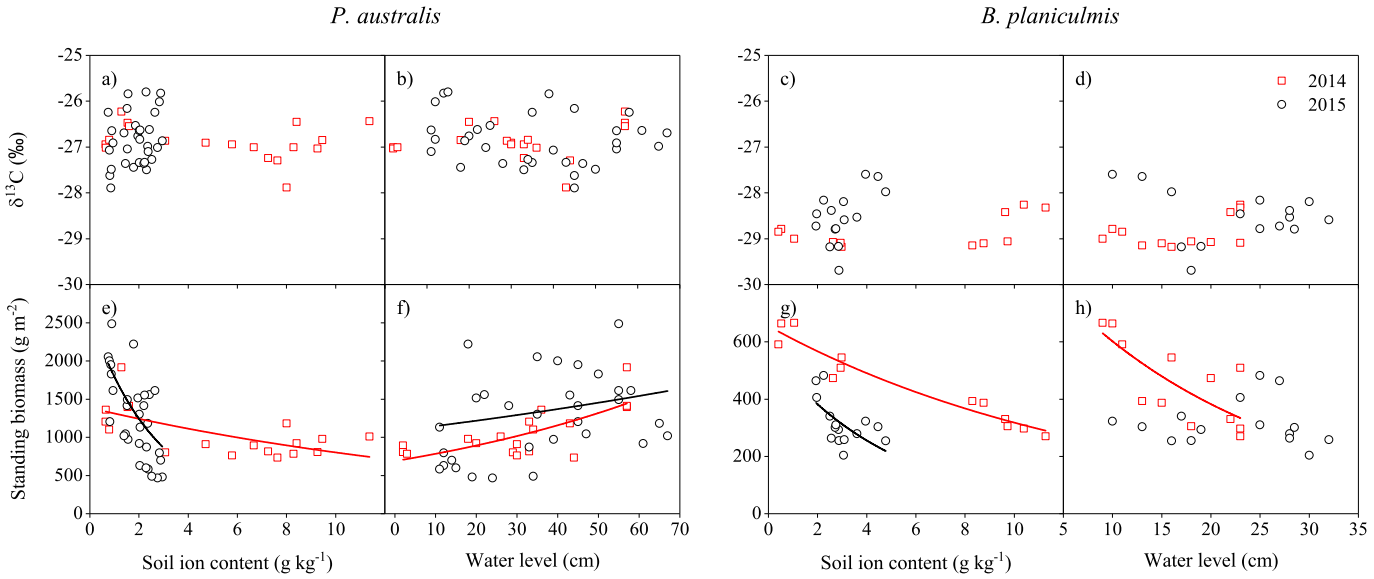

Fig. 4. Foliar $\delta^{13}C$ and standing biomass of *P. australis* and *B. planiculmis* in relation to soil ion content (a, c, e, g) and water level (b, d, f, h) in the field in 2014 (red squares) and 2015 (black circles) (See Appendix A. for more specific details on the equations of lines in Table S1).

examine differences between the ion contents within the same water level, and differences between the stress types within the same ion content at the same water level. In order to meet the assumptions of homoscedasticity, standing biomass was log-transformed.

3. Results

3.1. Soil ion and flooding characteristics in the field

Overall, the soil ion content of the 22 plots varied between 0.66 g kg^{-1} and 10.44 g kg^{-1}, with a median content of 2.50 g kg^{-1} (Fig. 2). HCO_3^- was the most abundant ion (median content = 1.38 g kg^{-1}, 0.35–5.65 g kg^{-1}), followed by Na^+ 0.54 g kg^{-1}, ranging from 0.04 to 2.67 g kg^{-1}, while the lowest values were found for K^+ and SO_4^{2-}. The water level ranged from 2 cm to 67 cm, with a mean of 29 cm and was negatively related to the soil ion content in 2015 ($r = -0.58$, $P < 0.001$, Fig. 3).

3.2. Response of foliar $\delta^{13}C$ and standing biomass of P. australis to soil ion content, water level and stress type

In the field, foliar $\delta^{13}C$ of *P. australis* was similar in the plants grown in soils of different ion contents and water levels (Fig. 4a, b; Table 1). However, standing biomass of *P. australis* decreased significantly with higher soil ion content (Fig. 4e; Table 1), but increased with water level (Fig. 4f; Table 1) in both years. In the greenhouse, the relationships were similar to those in the field (Fig. 5b, e, f; Table 2), except for a significant positive relationship between foliar $\delta^{13}C$ and soil ion content (Fig. 5a; Table 2). Soil ion content exerted the strongest influence on foliar $\delta^{13}C$ and standing biomass (Table 2).

In the greenhouse, the combination of low water levels (e.g. 0 cm) and high soil ion content (e.g. 10.0 or 20.0 g kg^{-1}) exerted a negative synergistic effect on the growth of *P. australis*, (Table 1, Fig. 6). Particularly, at 0 cm water level, *P. australis* was not able to grow in 5 to 20.0 g kg^{-1} alkaline ion contents and in 10 to 20 g kg^{-1} of saline-alkaline ion contents (Fig. 6d, f). In saline treatments, on the other hand, *P. australis* was able to grow across all ion contents (Fig. 6e). There was no big difference in standing biomass in 2.5 and 5.0 g kg^{-1} ion contents across all three stress types (Fig. 6). For plants grown under high ion content (10.0 g kg^{-1}), alkaline stress had the largest effect on foliar $\delta^{13}C$, whereas for plants grown under low ion contents (0, 2.5 or 5.0 g kg^{-1}), there was no significant difference between the three stress types (Fig. 6a, b, c).

3.3. Response of foliar $\delta^{13}C$ (WUE) and standing biomass of B. planiculmis to soil ion content, water level and stress type

In the field and the greenhouse, foliar $\delta^{13}C$ of *B. planiculmis* increased and standing biomass decreased with increasing ion contents, similar to

Table 1
Linear mixed-effect model results of the effects of soil ion content (SIC), water level (WL) and species (SP) on foliar $\delta^{13}C$ and standing biomass of *P. australis* and *B. planiculmis* in the field in 2014 and 2015. Spatial variations of sites are thus represented by the two fixed effects SIC and WL.

Year	Model	Foliar $\delta^{13}C$					Model	Standing biomass				
		Estimate	SE	df	t-Value	P-value		Estimate	SE	df	t-Value	P-value
2014	SIC	−0.01	0.03	21.99	−0.24	0.815	SIC	−0.01	0.01	20.86	−1.14	0.266
	WL	0.07	0.02	18.59	3.71	0.002	WL	−0.00	0.00	20.72	−1.21	0.238
	SP	2.51	0.32	17.17	7.73	<0.001	SP	−0.13	0.08	16.11	−1.63	0.122
	SIC:WL	−0.00	0.00	22.01	−1.47	0.160	SIC:WL	−0.00	0.00	19.97	−3.83	0.001
	SIC:SP	0.04	0.04	18.33	1.11	0.283	SIC:SP	0.03	0.01	22.02	3.69	0.001
	WL:SP	−0.05	0.01	14.38	−3.40	0.004	WL:SP	0.01	0.00	16.37	3.60	0.002
2015	SIC	0.56	0.28	33.60	1.99	0.055	SIC	−0.19	0.08	39.20	−2.31	0.026
	WL	0.06	0.04	27.72	1.61	0.119	WL	−0.03	0.01	39.89	−2.17	0.036
	SP	2.29	0.79	14.73	2.90	0.011	SP	0.72	0.31	36.03	2.36	0.024
	SIC:WL	−0.01	0.01	25.85	−1.55	0.134	SIC:WL	0.01	0.00	39.30	2.19	0.034
	SIC:SP	0.12	0.18	6.29	0.66	0.534	SIC:SP	−0.26	0.09	31.71	−2.94	0.006
	WL:SP	−0.04	0.03	30.03	−1.31	0.200	WL:SP	0.01	0.01	39.59	1.71	0.095

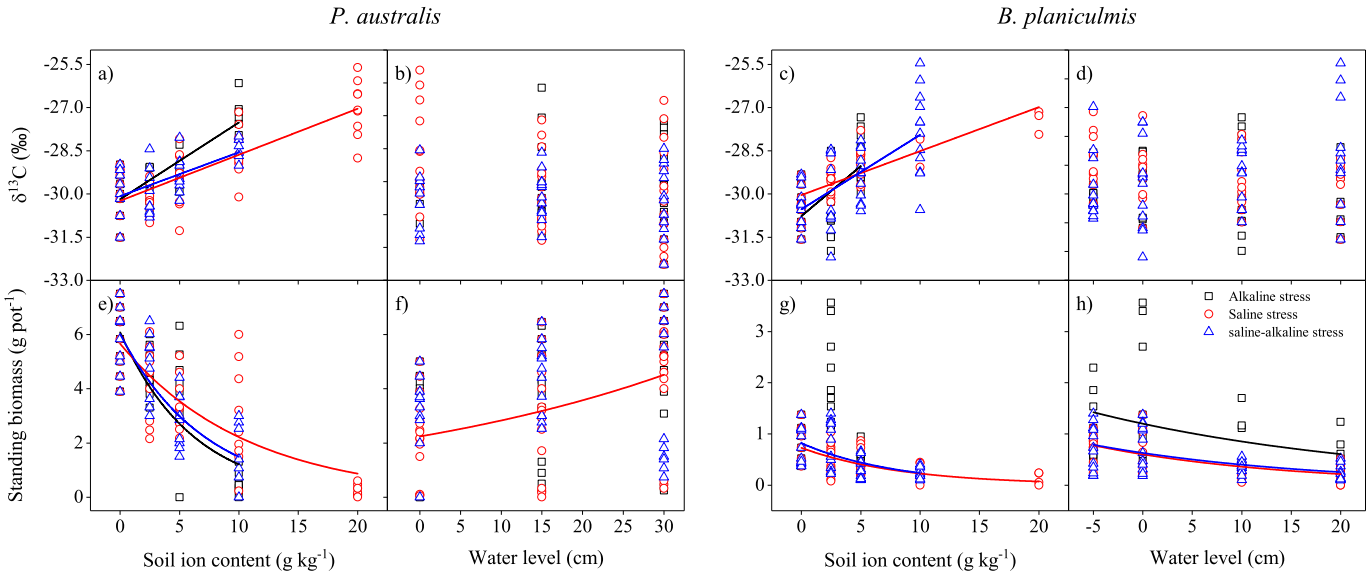

Fig. 5. Foliar $\delta^{13}C$ and standing biomass of *P. australis* and *B. planiculmis* in relation to soil ion content (a, c, e, g) and water level (b, d, f, h) of plants grown in the greenhouse under alkaline stress (black squares), saline stress (red circles) and saline-alkaline stress (blue triangles) (See Appendix A. for more specific details on the equations of lines in Table S2).

P. australis (Figs. 4c, d, g, 5c, d, g; Table 1), except for a significant negative relationship between standing biomass and water level (Figs. 4h, 5h; Table 1). Soil ion content exerted the strongest influence on foliar $\delta^{13}C$ and standing biomass (Table 2).

In the greenhouse, the combination of high water level (10 or 20 cm) and high soil ion content (5.0, 10.0 or 20.0 g kg^{-1}) had a negative synergistic effect (Table 2, Fig. 7). *B. planiculmis* was not able to grow in the 10.0 to 20.0 g kg^{-1} ion contents under alkaline stress and in the 20.0 g kg^{-1} ion content under saline-alkaline stress treatment (Fig. 7d, f). In contrast, it was able to grow across all ion contents in the saline stress treatment, except at 10.0 to 20.0 g kg^{-1} ion contents at 20 cm water level (Fig. 7e). However, standing biomass of *B. planiculmis* was significantly higher in 2.5 g kg^{-1} ion content than that in 0 and 5.0 g kg^{-1} ion contents under alkaline stress (Fig. 7d). In 2.5 g kg^{-1} ion content, foliar $\delta^{13}C$ was lower and standing biomass and higher under alkaline stress, compared to saline and saline-alkaline stress. There was no large difference in standing biomass in the 5.0 g kg^{-1} ion content across the three stress types (Fig. 7).

3.4. Comparison of the species responses to environmental effects in the field

Overall, foliar $\delta^{13}C$ values of *P. australis* were significantly higher than those of *B. planiculmis*, while standing biomass was much lower for *B. planiculmis* than for *P. australis* (Fig. 4a, c; Table 1).

4. Discussion

4.1. Variations of soil ion content and water level in the field

According to the Chinese grading standard for salinized soil (Soil fertilizer station of Jilin Province, 1998), five of our sites show no salinization problem (<1 g kg^{-1}), while fourteen of them show slight salinization (1–3 g kg^{-1}), three show moderate salinization (3–5 g kg^{-1}), and five show severe (>5 g kg^{-1}) salinization. This suggests that Western Songnen plain wetlands are mainly suffering from slight saline-

Table 2
Linear mixed-effect model results of the effect of soil ion content (SIC), water level (WL) and stress type (ST) on foliar $\delta^{13}C$ and standing biomass of *P. australis* and *B. planiculmis* in the greenhouse.

Species	Model	Foliar $\delta^{13}C$				Model	Standing biomass			
		DF	MS	f-Value	P-value		DF	MS	f-Value	P-value
P. australis	SIC	1	81.85	158.99	<0.001	SIC	1	4.54	200.16	<0.001
	WL	1	1.48	2.88	0.094	WL	1	0.78	34.25	<0.001
	ST	3	2.95	5.73	0.001	ST	3	0.18	7.97	<0.001
	SIC:WL	1	0.10	0.20	0.657	SIC:WL	1	0.00	0.02	0.896
	SIC:ST	2	3.00	5.83	0.004	SIC:ST	2	0.26	11.62	<0.001
	WL:ST	3	1.59	3.09	0.032	WL:ST	3	0.03	1.10	0.353
	SIC:WL:ST	2	0.27	0.51	0.600	SIC:WL:ST	2	0.02	0.85	0.429
B. planiculmis	SIC	1	63.40	99.25	<0.001	SIC	1	0.41	115.85	<0.001
	WL	1	2.42	3.78	0.055	WL	1	0.38	106.02	<0.001
	ST	3	0.38	0.59	0.622	ST	3	0.09	25.47	<0.001
	SIC:WL	1	3.01	4.71	0.033	SIC:WL	1	0.03	8.01	0.006
	SIC:ST	2	8.76	13.72	<0.001	SIC:ST	2	0.18	51.64	<0.001
	WL:ST	3	5.51	8.63	<0.001	WL:ST	3	0.00	0.38	0.769
	SIC:WL:ST	2	1.69	2.65	0.077	SIC:WL:ST	2	0.02	5.52	0.006

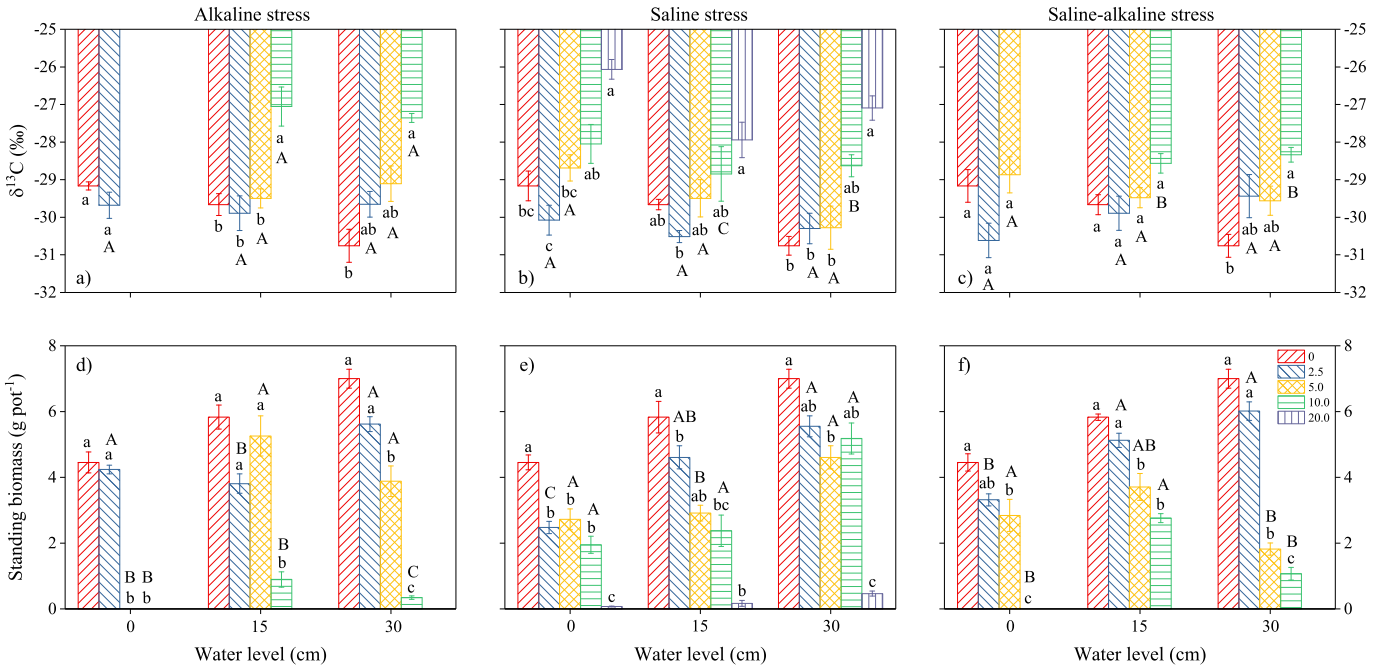

Fig. 6. Effects of alkaline (a, d), saline (b, e), saline-alkaline stress (c, f) and water stress (0, 15 and 30 cm) on foliar $\delta^{13}C$ and standing biomass of *P. australis* grown at different soil ion contents (colored bars) in the greenhouse (means ± 1 s.e., n = 3). Different lowercase letters indicate significant differences between soil ion contents within each water level ($P < 0.05$). Different capital letters indicate significant differences between stress types within each soil ion content at the same water level ($P < 0.05$). A Tukey *post-hoc* test was used for multiple comparisons.

alkalization stress. HCO_3^- and Na^+ showed the highest ion contents (Fig. 2), indicating that sodium bicarbonate ($NaHCO_3$) is the main component of salinization in the wetlands of Western Songnen Plain.

We observed a negative relationship between water level and soil ion content (Fig. 3). In Western Songnen Plain, groundwater is high in soluble salts such as sodium and potassium carbonates that are derived from parent materials which contain abundant sodium aluminosilicates (Lin and Tang, 2005). If groundwater rises to the soil surface and inundates the soil to a certain level, these soluble salts are diluted and can also be transported away by the adjacent river. On the other hand,

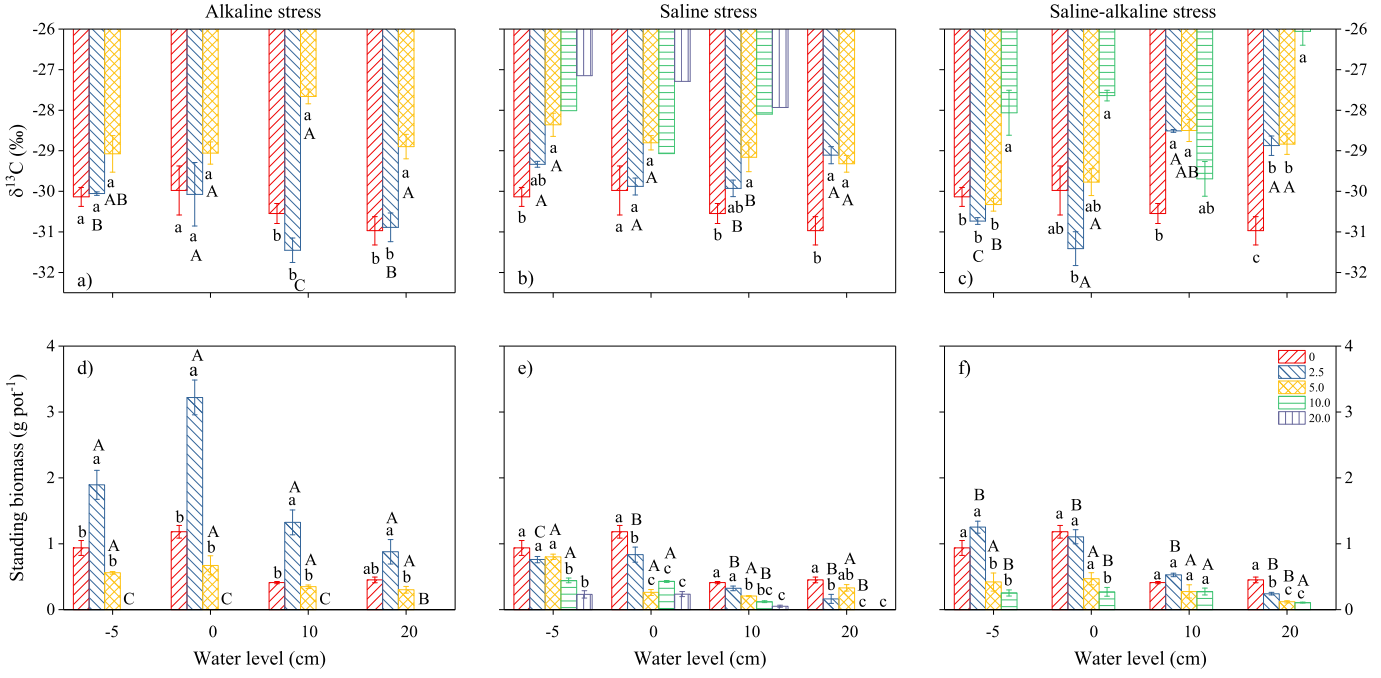

Fig. 7. Effects of alkaline (a, d), saline (b, e), saline-alkaline stress (c, f) and water stress (−5, 0, 10 and 20 cm) on foliar $\delta^{13}C$ and standing biomass of *B. planiculmis* grown at different soil ion contents (colored bars) in the greenhouse (means ± 1 s.e., n = 3). Different lowercase letters indicate significant differences between soil ion contents within each water level ($P < 0.05$). Different capital letters indicate significant differences between stress types within each soil ion content ($P < 0.05$). In the 10 and 20 g kg^{-1} saline treatments we combined the three replicates for foliar $\delta^{13}C$ measurements due to low leaf dry mass. Consequently, no error bars are provided for these samples. A Tukey *post-hoc* test was used for multiple comparisons.

Table 3
Means (±1 s.e.) of foliar $\delta^{13}C$ of *P. australis* and *B. planiculmis* in the field compared with the species in salt marshes in northern China.

Species	Foliar $\delta^{13}C$ (‰)	Soil ion content (g kg^{-1})	Location	Reference
P. australis	−25.80 ± 0.21	4.45 ± 1.04	Tianjin	Wang (2012)
P. australis	−26.92 ± 0.07	3.29 ± 0.31	Western Songnen Plain	This study
P. australis	−27.67 ± 0.47	1.83 ± 0.30	Yellow River Delta	Chen et al., 2015
B. planiculmis	−28.67 ± 0.11	3.29 ± 0.31	Western Songnen Plain	This study
Tamarix chinensis	−28.99 ± 0.47	1.83 ± 0.30	Yellow River Delta	Chen et al. (2015)
Suaeda glauca	−29.45 ± 0.29	1.83 ± 0.30	Yellow River Delta	Chen et al. (2015)

soluble salts will accumulate on the soil surface due to strong evaporation, which tends to result in high saline-alkaline stressed soil. Thus, low water levels result in high soil ion contents.

4.2. Influence of soil ion content and water level on foliar WUE and standing biomass

P. australis and *B. planiculmis* showed substantially higher WUE (except in the field, Fig. 4a) and lower standing biomass with increasing soil ion content (Fig. 4c, e, g; Fig. 5a, c, e, g). Similar results have also been demonstrated by other studies (Brugnoli and Lauteri, 1991; Choi et al., 2005; Gorai et al., 2011; Hellings and Gallagher, 1992; Wei et al., 2008). However, three reasons might contribute to the fact that foliar $\delta^{13}C$ was not related to soil ion contents in the field: (1) the lack of data for high soil ion contents; (2) the much lower range in soil ion contents in the field compared to the greenhouse; and (3) additional factors contributing to variations in plant growth in the field compared to the controlled conditions in the greenhouse. Stomatal conductance and photosynthetic capacity are the main factors that affect WUE (Brugnoli and Lauteri, 1991). Higher WUE as indicated by high $\delta^{13}C$ values, suggests a lower C_i/C_a (C_i is the intercellular CO_2 concentration) and thus either a lower stomatal conductance or a higher photosynthesis rate. Lower standing biomass indicates that the photosynthesis rate was not elevated, but instead, higher WUE was most probably due to reduced stomatal conductance under high soil ion content. High soil ion contents also inhibit K^+ uptake, induce oxidative stress and high pH stress on plants and thus inhibit growth and development (Agarwal and Pandey, 2004; Ghoulam et al., 2002; Upadhyay and Panda, 2005).

High water levels increased standing biomass of *P. australis* (Figs. 4f, 5f), while it reduced standing biomass of *B. planiculmis* (Figs. 4h, 5h). This pattern was consistent with those observed in previous studies (Coops et al., 1996; Hellings and Gallagher, 1992; Liu et al., 2016). Gafny and Gasith (1999) found that there was "a window of opportunity" for wetland plant growth, that is, within a certain range of water levels, plants benefit and reach the maximum standing biomass. As *P. australis* has a very efficient pathway to supply oxygen to its belowground structures (Ailstock and Center, 2000), the optimal water level is higher than that of *B. planiculmis*. Thus, standing biomass of *P. australis* increased and *B. planiculmis* decreased within 30 cm water level.

Our greenhouse study showed that high soil ion content exerted a stronger influence on WUE and standing biomass of *P. australis* and *B. planiculmis* than the water level and the stress type (Table 2). Saline ion content generates more stresses than water level: besides oxygen deficit, osmotic stress and oxidative stress that are already exerted by water stress (Ahmad et al., 2011; Smirnoff, 1993), high saline ion contents also exert ionic toxicity and membrane dysfunction on plants, which can destroy root structures and functions, leading to cell death (Agarwal and Pandey, 2004; Ghoulam et al., 2002; Upadhyay and Panda, 2005). Moreover, high alkaline ion content (especially 10 g kg^{-1}) had a particularly large effect on WUE and standing biomass of the two species (Figs. 6, 7). High alkaline ion contents exert the same detrimental effects as saline stress, but in addition, also decreases the availability of mineral nutrients, and directly damages membrane structure and function (i.e. Na^+ absorption) of root cells (Li et al., 2008; Yang et al., 2006; Zhang et al., 2008). Thus, high soil alkalinity is more harmful for plant growth than high salinity and water stress. However, alkaline conditions promoted the growth of *B. planiculmis* under low ion content (2.5 g kg^{-1} alkaline ion content; Fig. 7). The different responses of the two species are not fully understood, but may be due to different sensibilities to soil alkaline ion content and nutrient imbalances (Bailey, 1996). In addition, the negative effect of high soil ion contents increased with decreasing water levels on the growth of *P. australis*, while it increased with increasing water levels on the growth of *B. planiculmis*, even leading to plant death at very low or high water levels, respectively (0 or 20 cm water level; Fig. 6d, e, f; Fig. 7d, e, f). This suggests that the tolerance of *P. australis* and *B. planiculmis* to high soil ion contents is lost when suffering drought and flooding stress, respectively. Hence, high soil ion content was the main factor influencing wetland plant growth and had a negative synergistic effect with drought on the survival of *P. australis* and with flooding on the survival of *B. planiculmis*.

4.3. Comparison of WUE and standing biomass between wetland species

P. australis and *B. planiculmis* showed different adaptive strategies to saline-alkaline and water stresses. *P. australis* was more tolerant to high soil ion contents than *B. planiculmis*, particularly under alkaline conditions (Fig. 6d, 7d). That may be related to the fact that compared to *B. planiculmis*, *P. australis* is a pseudohalophyte which has the ability to keep most of the Na^+ absorbed from soil in roots to reduce the toxic effect on its aboveground part (Zhao et al., 1998). On the other hand, *B. planiculmis* grew well under low water level conditions (<10 cm), while *P. australis* preferred high water levels. The two species differed to a great extent in their WUE (Tables 1, 3). Our analysis also showed that WUE of *P. australis* in the field in Tianjin was significantly higher than in the field in our study and the Yellow River Delta which are both characterized by lower soil ion contents than Tianjin (Table 3). This supports our explanation that high soil ion contents resulted in high WUE. Relative to other wetland plants, WUE of *P. australis* was the highest (Table 3), indicating that *P. australis* had a high ability to modulate and increase WUE to resist its adverse environment.

5. Conclusions

Our findings indicate that the tolerance of *P. australis* to high soil ion contents is stronger than that of *B. planiculmis*, but when suffering from drought, the capacity of *P. australis* to tolerate high soil ion contents (especially alkaline stress) is largely lost. *P. australis* has a high ability to modulate and increase WUE to resist its adverse environment. Our study will contribute to a better understanding of the influence of climate change and increasingly serious human disturbances on the distribution and productivity of these two important wetland species.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.05.321.

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